MAJOR ARTICLE

Interferon (IFN)–γ–Inducible Protein–10: Association with Histological Results, Viral Kinetics, and Outcome during Treatment with Pegylated IFN-α2a and Ribavirin for Chronic Hepatitis C Virus Infection

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Background. We investigated associations between interferon (IFN)–γ–inducible protein (IP)–10 and liver histological results, viral kinetic response, and treatment outcome in patients infected with hepatitis C virus (HCV) genotypes 1–4.

Methods. Plasma IP-10 was monitored before, during, and after treatment with pegylated IFN-α2a and ribavirin in 265 HCV-infected patients.

Results. In univariate analyses, a low baseline IP-10 level was significantl associated with low baseline viral load, rapid viral response (RVR), a sustained viral response (SVR), body mass index <25 kg/m^2, and less-pronounced fib osis, inflammation and steatosis (for HCV genotypes other than 3). When the results of the univariate analyses were included in multivariate analyses, a low plasma IP-10 level, low baseline viral load, and genotype 2 or 3 infection were independent predictors of an RVR and SVR. IP-10 levels decreased 6 weeks into treatment and remained low in patients with an SVR. By contrast, plasma levels of IP-10 rebounded in patients who had detectable HCV RNA after the completion of treatment. Using cutoff IP-10 levels of 150 and 600 pg/mL for predicting an SVR in patients infected with HCV genotype 1 yielded a specificit and sensitivity of 81% and 95%, respectively.

Conclusion. Baseline IP-10 levels are predictive of the response to HCV treatment.

Hepatitis C virus (HCV) is the major cause of parenterally transmitted non-A, non-B hepatitis [1, 2] and is associated with chronic hepatitis, cirrhosis, and hepatocellular carcinoma [3, 4]. With currently available treatment with pegylated (PEG)–interferon (IFN)–α and ribavirin, a sustained viral response (SVR) is achieved in 50%–80% of infected individuals after the completion of treatment [5–7]. Factors independently associated with a favorable outcome after therapeutic intervention include infection with an HCV genotype other than 1, viral loads $<2 \times 10^6$ copies/mL, a body surface area <2 m^2, age <40 years, the absence of bridging fib osis or cirrhosis in pretreatment liver-biopsy samples, and a rapid viral response (RVR) after the onset of treatment [5–10].

The 10-kDa IFN-γ–inducible protein (IP-10 or CXCL10) is a chemotactic CXC chemokine of 77 aa in

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Table 1. Baseline characteristics of the patients, grouped by interferon-γ–inducible protein (IP)–10 level before treatment.

Characteristic	IP-10 level		
	<150 pg/mL	150–600 pg/mL	>600 pg/mL
Age, mean ± SD, years	38.9 ± 8.3	42.6 ± 10.7	42.5 ± 10.5
Sex, no. (%)			
Male	62 (77)	102 (67)	14 (44)
Female	19 (23)	50 (33)	18 (56)
BMI, mean ± SD, kg/m^2	24.5 ± 3.6	25.3 ± 3.3	25.8 ± 4.8
HCV genotype, 1/2/3/4/5	47/10/18/5/1	102/13/29/8/0	24/1/6/1/0
HCV-RNA load, mean ± SD, $\log_{10}$ IU/mL	6.0 ± 0.7	6.1 ± 0.7	6.4 ± 0.6
Normalized ALT (ALT/ULN), mean ± SD	2.1 ± 1.5	2.5 ± 0.2	3.1 ± 1.9
Normalized AST (AST/ULN), mean ± SD	1.1 ± 0.6	1.9 ± 1.5	2.4 ± 1.7
Ishak protocol scores			
Fibrosis stage, 0/1/2/3/4/5/6	3/34/18/8/5/4/1	8/26/40/16/14/13/9	1/4/9/6/1/3/5
Interface hepatitis grade, 0/1/2/3/4	8/36/23/6/0	7/50/45/24/0	1/7/14/6/0
Focal inflammation grade, 0/1/2/3/4	0/17/44/12/0	2/20/69/35/0	0/5/13/10/0
Portal inflammation grade, 0/1/2/3/4	3/44/28/1/0	4/47/62/13/0	1/7/17/3/0
Steatosis grade, 0/1/2/3	37/23/9/4	56/49/19/2	10/13/3/2

NOTE. Ishak protocols are described in [31]. ALT, alanine aminotransferase; ASP, aspartate aminotransferase; HCV, hepatitis C virus; ULN, upper limit of normal.

its mature form [11, 12]. Unlike other CXC chemokines, IP-10 lacks chemotactic activity for neutrophils—rather, it appears to target T lymphocytes, NK cells, and monocytes [12–14] through its receptor, CXCR3 [15, 16]. IP-10 can be produced by a variety of cells, including hepatocytes [17, 18], and it has been implicated in the pathophysiological progression of multiple sclerosis [19, 20], diabetes mellitus [21, 22], and HIV [23, 24]. In the setting of HCV infection, IP-10 mRNA expression in the liver has been reported to be associated with the presence of lobular necroinflammato y activity in liver-biopsy samples but not with portal inflammatio or fib osis [18]. Recently, baseline levels of IP-10 before the initiation of therapeutic intervention for HCV infection were reported to be higher in patients who did not achieve an SVR after the completion of treatment [25, 26], especially in the context of HCV genotype 1 infection. The aim of the present study was to investigate plasma IP-10 levels in patients with chronic HCV infection before, during, and after combination treatment with PEG–IFN-α2a and ribavirin and to examine possible associations with pretreatment liver histological results, initial viral kinetic reductions during treatment, and fina treatment outcome in patients infected with HCV genotypes 1, 2, 3, and 4, as well as to propose possible cutoff values for pretreatment IP-10 levels for the prognostication of fina treatment outcome.

PATIENTS, MATERIALS, AND METHODS

Patients. Between February 2001 and November 2003, 270 patients (180 men and 90 women) were recruited in a phase 3, open-label, randomized, multicenter trial conducted by the Dynamically Individualized Treatment of Hepatitis C Infection and Correlates of Viral/Host Dynamics study group at 9 centers in France, Germany, Greece, Israel, Italy, The Netherlands, Spain, Sweden, and Switzerland, as reported elsewhere [27]. All patients were adults (mean ± SD age, 41.6 ± 10.2 years), had compensated liver disease, were naive for hepatitis C treatment, and fulfille the following inclusion criteria: a positive test for anti-HCV antibodies, a viral load >1000 IU/mL, and 2 serum alanine aminotransferase values above the upper limit of normal within 6 months of the initiation of treatment. Baseline characteristics of the patients are shown in table 1.

Treatment. All patients were initially treated for 6 weeks with 180 μg of PEG–IFN-α2a subcutaneously once weekly (Pegasys; Hoffmann–La Roche) and ribavirin orally twice daily (Copegus; Hoffmann–La Roche) at a total daily dose of 1000 mg for patients weighing <75 kg and 1200 mg daily for those weighing ⩾75 kg. After 6 weeks of treatment, one-half of the patients were randomized on the basis of their viral kinetic classification as outlined below, to receive individualized treatment or to continue receiving standard combination treatment for a total of 48 weeks.

Viral loads. The plasma viral load was determined by reverse-transcription polymerase chain reaction using the Cobas Amplicor HCV Monitor (version 2.0; Roche Diagnostics) HCV RNA quantificatio was performed on days 0, 1, 4, 7, 8, 15, 22, and 29; at the end of treatment; and 24 months after the completion of treatment.

Classificatio of the viral kinetic response. Patients were classifie as rapid viral responders, slow partial responders, fla

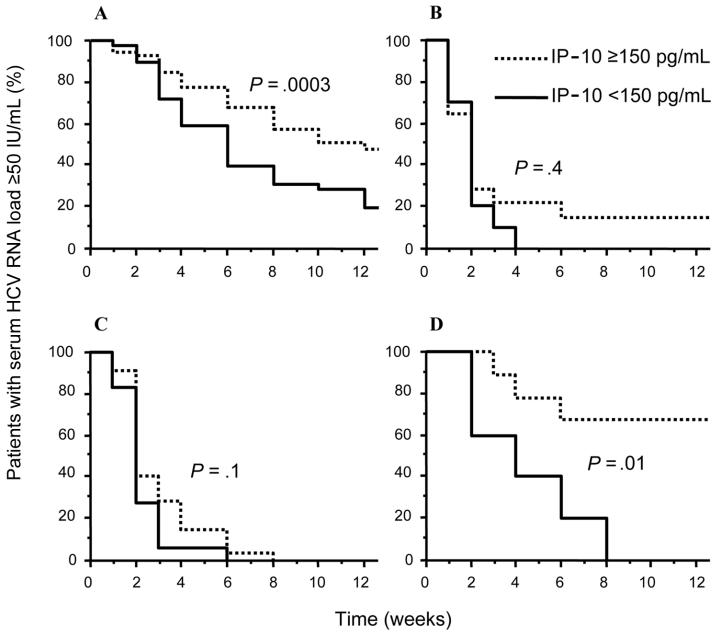

Figure 1. Kaplan-Meier curve of cumulative hepatitis C virus (HCV) RNA negativity (defined as plasma viral loads ≤50 IU/mL) during the first 12 weeks of treatment. Patients are grouped as having interferon-γ–inducible protein (IP)–10 concentrations ≥ or <150 pg/mL at baseline, before the onset of combination treatment with pegylated interferon-α2a and ribavirin for HCV genotype 1 ($n = 169$) *(A),* genotype 2 ($n = 24$) *(B),* genotype 3 ($n = 53$) *(C),* and genotype 4 ($n = 14$) *(D)* infection. *P* values were obtained using the log-rank test.

partial responders, or null responders, according to the individual decrease in serum viral loads during the firs 4 weeks of treatment; they were then randomized within each viral kinetic class either to receive an individualized treatment regimen or to a control group, as reported elsewhere [27]. An RVR was define as a ⩾2-log reduction in viral load during the firs 4 weeks of treatment and a second-phase decline of ⩾0.09/day. Investigators and patients were blinded to viral load results until the randomization after 6 weeks of treatment.

Classificatio of treatment outcome. Patients were classifie as having an SVR if the serum viral load was undetectable 24 weeks after the completion of treatment. Patients were classifie as having a relapse if the serum viral load was undetectable at end of treatment but detectable 24 weeks after the completion of treatment. Patients were classifie as nonresponders if HCV RNA was detectable in serum at the end of treatment and 24 weeks after the completion of treatment.

Genotyping. Genotyping of HCV was performed using the INNO-LiPA HCV II device (Innogenetics). In total, 178 patients were infected with HCV genotype 1, 24 with genotype 2, 53 with genotype 3, 14 with genotype 4, and 1 with genotype 5.

IP-10 quantification Human IP-10 was quantifie using Quantikine (R&D Systems)—a solid-phase ELISA—on plasma samples obtained during the week before the start of treatment, 6 weeks after the start of treatment, and 12 weeks after the completion of treatment. Of the 270 patients included in the study, plasma samples from 265 were available for analysis at baseline (i.e., before the onset of combination treatment), 254 were available 6 weeks after the start of treatment, and 80 were available for analysis 12 weeks after the completion of treatment. All samples were stored at −70°C until assay. When IP-10 levels in serum were analyzed in samples from 24 healthy, HCV-negative blood donors, a mean ± SD level of 87 ± 45 pg/mL (median, 76 pg/mL) was noted.

Liver biopsies. Liver-biopsy samples had been obtained from all patients within 12 months preceding their inclusion in the study. Only biopsies with a length ⩾1 cm and that contained at least 4 portal tracts were evaluated; the majority of samples had a length >2 cm and contained >6 portal tracts. In total, liver-biopsy samples from 231 patients were retrieved and evaluated. For each biopsy, a hematoxylin-eosin stain and a Sirius Red stain were centrally staged for fib osis and graded for interface hepatitis, confluen necrosis, focal inflammation and portal inflammatio by 2 independent observers (J.W. and M.L., under the supervision of A.P.D.) experienced in the pseudonumerical scoring of liver-biopsy samples with a documented acceptable interobserver variability [4, 28–30], in a blinded fashion, in accordance with the method of Ishak et al. (the Ishak protocol) [31]. Equivocal issues were debated after the independent scores were noted, and a consensus score was obtained. In addition, steatosis was graded as follows: 0, absent;

1, <30% of hepatocytes involved; 2, 30%–70% of hepatocytes involved; and 3, >70% of hepatocytes involved [30].

Statistical methods. Individual characteristics between groups were evaluated using the Wilcoxon–Mann-Whitney *U* test. Reductions in viral load were displayed in Kaplan-Meier cumulative survival plots in which survival was define as having a serum viral load >50 IU/mL (the limit of detection of the Cobas Amplicor HCV Monitor version 2.0), with the use of log-rank tests for comparisons between the Kaplan-Meier plots. Patients were excluded from this analysis for whom the time of the firs serum sample with a viral load <50 IU/mL could not be determined because of missing data ($n = 5$). Stepwise multivariate logistic-regression analyses were performed, using data from all patients in the study, for which the outcome variable was dichotomized as either having had an RVR during the firs 4 weeks of treatment (define as a ⩾2-log reduction in viral load during the firs 4 weeks of treatment) or not and achieving an SVR at follow-up week 24 or not. The following explanatory variables were analyzed in multivariate analyses: $\log_{10}$ plasma IP-10 level at baseline, $\log_{10}$ viral load at baseline, genotype, sex, body mass index (BMI), and age. Stepwise multivariate logistic-regression analysis was performed using the SAS software package (version 8; SAS Institute). All reported *P* values are 2-sided, and $P < .05$ was considered to indicate significance

Ethics approval. The treatment study was approved by ethics committees at each study center and conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Informed consent was obtained from each patient included in the study.

RESULTS

A baseline plasma IP-10 level <150 pg/mL (approximately equal to 2 SDs above mean IP-10 levels in HCV-negative blood donors) was significantl associated with an RVR during the firs 12 weeks of combination treatment in patients infected with HCV genotypes 1 ($P = .0003$, log-rank test) and 4 ($P = .01$, log-rank test), compared with patients who had baseline plasma IP-10 levels ⩾150 pg/mL. There was also a nonsignifican trend toward a faster clearance of HCV RNA in serum among the patients infected with genotypes 2 and 3 and who had lower baseline plasma IP-10 levels (figu e 1).

Figure 2 shows infected plasma IP-10 levels before, during, and after treatment in patients grouped as infected with HCV genotypes 1 and 4 (figu e 2*A*) or genotypes 2 and 3 (figu e 2*B*). IP-10 levels at baseline were significantl lower in patients infected with genotypes 1 and 4 who achieved an SVR than in those who did not ($P < .0001$). By contrast, no such difference was observed in patients infected with genotypes 2 and 3. After 6 weeks of treatment, IP-10 levels were reduced by 100–200 pg/mL in most patients, regardless of HCV genotype, even among nonresponders. Twelve weeks after the completion of treatment, IP-10 levels remained low in patients who achieved an SVR; however, among those who relapsed and among nonresponders, IP-10 levels rebounded to pretreatment levels. The reduction in IP-10 levels between baseline values and 12 weeks after the completion of treatment was statistically significan when patients who achieved an SVR were compared with those who did not, irrespective of HCV genotype ($P = .0002$) (figu e 2).

As seen in figu e 3, there was a trend toward lower baseline

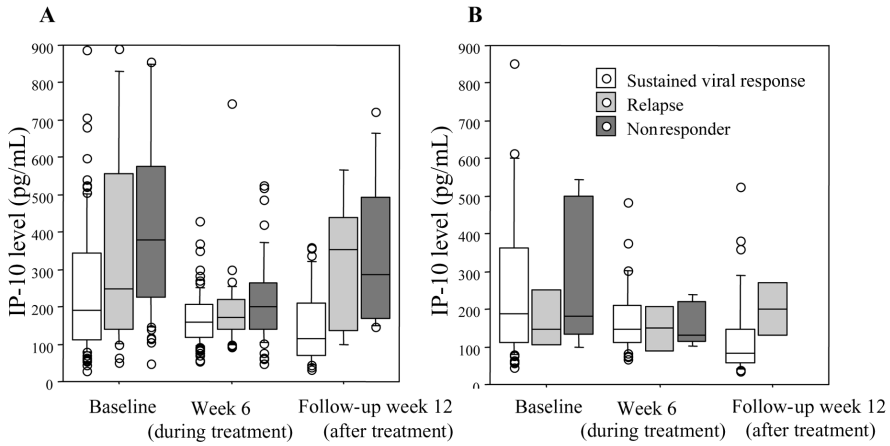

Figure 2. Box plot displaying the 10th, 25th, 50th, 75th, and 90th percentiles of interferon-γ–inducible protein (IP)–10 levels. Levels were determined at baseline, before the onset of combination treatment with pegylated interferon-α2a and ribavirin, 6 weeks after starting treatment and 12 weeks after the completion of treatment, in relation to the final treatment outcome. Patients were grouped by infection hepatitis C virus genotypes 1 and 4 *(A)* or genotypes 2 and 3 *(B)*.

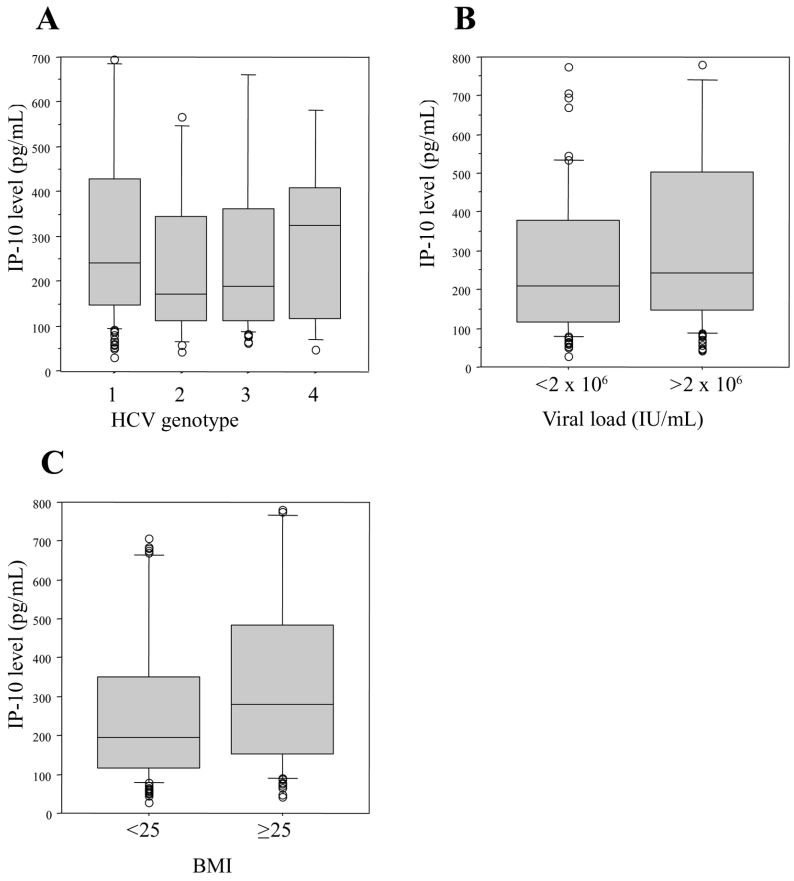

Figure 3. Box plot displaying the 10th, 25th, 50th, 75th, and 90th percentiles of interferon-γ–inducible protein (IP)–10 levels. Levels were determined at baseline, before the onset of combination treatment with pegylated interferon-α2a and ribavirin, in relation to hepatitis C virus (HCV) genotype *(A),* baseline viral load *(B),* and body mass index (BMI) *(C).*

IP-10 levels in patients infected with HCV genotypes 2 and 3, compared with patients infected with HCV genotypes 1 and 4, although this was not statistically significan ($P = .1$). A significan association, however, was noted between the baseline viral load before the initiation of antiviral treatment and the baseline IP-10 level. Patients with baseline viral loads $>2 \times 10^6$ IU/mL thus had significantl higher IP-10 levels than those with lower viral loads for all genotypes, especially those infected with genotype 1 (for all genotypes, $P = .05$; for genotype 1, $P < .0001$). Likewise, patients with a BMI ≥ 25 kg/m^2 (the median BMI in the study) had significantl higher IP-10 levels at baseline than those with a BMI <25 kg/m^2 ($P = .009$). Interestingly, neither body weight above or below the median of 75 kg nor height greater than or less than the median of 172 cm was independently associated with IP-10 levels ($P = .4$ and $P = .1$, respectively). Similarly, age above or below 40 years was not significantl associated with IP-10 levels.

The association between liver histological results and IP-10 levels at baseline is illustrated in figu e 4. There was a trend toward increasing IP-10 levels with increasing Ishak fib osis stage; however, a considerable overlap between fib osis stages was observed. Statistically significantl higher plasma IP-10 levels were seen in patients with bridging fib osis, compared with those without ($P = .009$) and in patients with versus without cirrhosis ($P = .03$). Statistically significan associations between IP-10 levels and the interface hepatitis score (interface score ≤ 2 vs. 3, $P = .0002$), the focal inflammatio score (focal score ≤ 2 vs. 3, $P = .0015$), and the portal inflammatio score (portal score ≤ 1 vs. ≥ 2, $P = .0001$) were observed. As is shown, a significan association between steatosis grade >1 (i.e., involving >30% of hepatocytes) was seen in patients infected with HCV genotypes other than 3 ($P = .01$). In patients infected with genotype 3, a nonsignifican trend was observed toward higher baseline IP-10 levels with increasing steatosis grade ($P = .3$).

To determine whether IP-10 is an independent predictor of the initial viral response and the fina treatment outcome, stepwise multivariate logistic-regression analyses were performed using plasma IP-10 levels at baseline, viral load at baseline, genotype, sex, BMI, and patient age as potential explanatory variables. As seen in table 2, when the outcome variable was

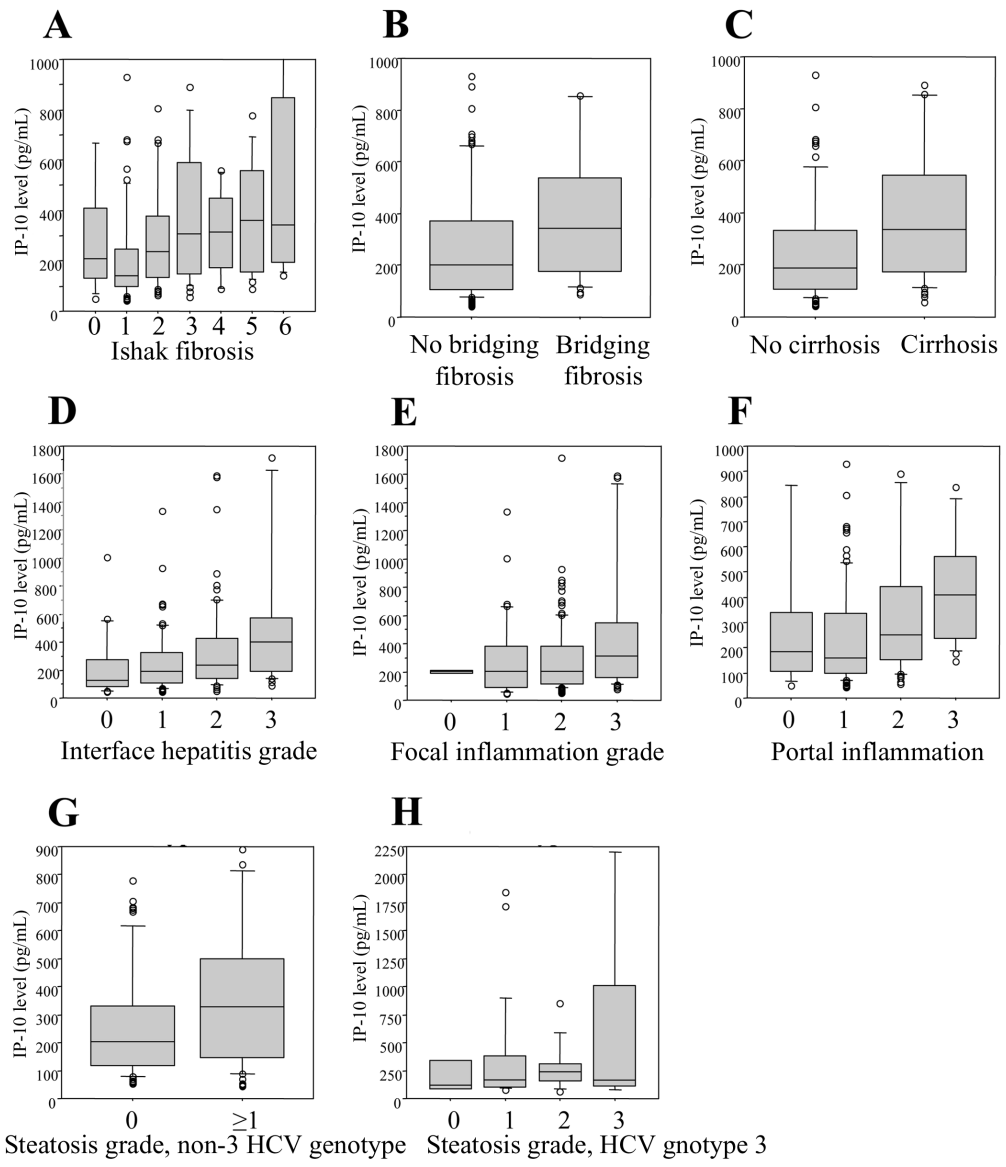

Figure 4. Box plot displaying the 10th, 25th, 50th, 75th, and 90th percentiles of the interferon-γ–inducible protein (IP)–10 levels. Levels were determined at baseline, before the onset of combination treatment with pegylated (PEG)–interferon-α2a and ribavirin, in relation to Ishak fibrosis stage [31] *(A),* the presence or absence of bridging fibrosis (Ishak stage ⩾3) *(B),* the presence or absence of cirrhosis (Ishak stage ⩾5) *(C),* Ishak interface hepatitis grade *(D),* Ishak focal inflammation grade *(E),* Ishak portal inflammation grade *(F),* steatosis grade for hepatitis C virus (HCV) genotypes other than 3 *(G),* and steatosis grade for genotype 3 *(H).*

dichotomized as either having an RVR during the firs 4 weeks of treatment or not (define as a ⩾2 log reduction in serum viral load), baseline IP-10 level, baseline viral load, and genotype were independent significan explanatory variables.

When the outcome variable in the stepwise multivariate logistic-regression analysis was dichotomized as either having an SVR or not at the end of follow-up, the significan independent explanatory variables were baseline IP-10 level, baseline viral load, patient age, and genotype. As was the case with the initial viral response, genotype was the strongest predictor of fina treatment outcome, with HCV genotype 3 having the highest odds ratio (OR). However, baseline IP-10 levels were associated with a higher OR than baseline viral load and patient age (table 3). IP-10 and genotype remained independent predictors of an RVR when fib osis stage, interface hepatitis grade, focal inflam mation grade, portal inflammatio grade, and steatosis grade were included in the multivariate analysis (data not shown). Similarly, IP-10 level, baseline viral load, and genotype remained independent predictors of an SVR, whereas age was replaced by fib osis stage when the liver-biopsy scores were

Table 2. Multivariate logistic-regression to identify factors predictive of not achieving a rapid viral response ($n = 263$).

Parameter	Regression coefficient	SE	Odds ratio	*P*
Baseline $\log_{10}$ IP-10 level, pg/mL	2.64	0.52	13.987	<.0001
Baseline $\log_{10}$ viral load, IU/mL	0.53	0.25	1.701	.033
HCV genotype				
1	1.28	0.34	0.627	.0002
2	−1.10	0.67	0.058	.1006
3	−1.93	0.58	0.025	.0008
Constant	−10.96	2.02		<.0001

NOTE. IP-10, interferon-γ–inducible protein.

included. However, because not all patients provided liver-biopsy samples, the predictive models generated were not as well fitte as when liver histological scores were excluded.

When we used cutoff IP-10 levels of 150 and 600 pg/mL for patients infected with HCV genotype 1, the specificit was 81% (positive predictive value, 68%) and the sensitivity was 95% (negative predictive value, 79%). In other words, ~7 of 10 genotype 1–infected patients who had an IP-10 level ≤150 pg/mL before the onset of antiviral treatment achieved an SVR after the completion of treatment, compared with only 2 of 10 patients who had an IP-10 level >600 pg/mL. The predictive value of the IP-10 level was similar, irrespective of whether patients had received standard or individualized treatment (data not shown).

DISCUSSION

In the present study, we noted a relatively strong association between low baseline plasma IP-10 levels and a favorable viral kinetic response during combination treatment with PEG–IFN-α2a and ribavirin in patients infected with HCV genotypes 1 and 4. A similar association was observed between low IP-10 levels and SVR. In multivariate analyses, the baseline IP-10 level was independently predictive of both RVR and the fina treatment outcome. However, in both of these analyses, having HCV genotype 2 or 3 infection was associated with higher ORs than baseline IP-10 levels, which indicates that genotype remains the strongest predictor of the initial viral response and of the fina treatment outcome. The OR observed for baseline IP-10 level, however, was higher than that observed for baseline viral load with regard to initial viral response and also was higher than those noted for baseline viral load and patient age with regard to SVR. Taken together, these data may suggest that IP-10 plays a role as a readily obtainable baseline variable for the prediction of an RVR and of the f nal treatment outcome in patients infected with HCV genotypes 1 and 4.

As seen in figu e 2, there was considerable overlap in baseline IP-10 levels among the patients infected with HCV genotypes 1 and 4 who achieved an SVR after the completion of treatment, compared with those who did not. However, when we used cutoff levels of 150 and 600 pg/mL for patients infected with HCV genotype 1, the positive predictive value was 68% and the negative predictive value was 79%. It should, however, be noted that 59% of the patients infected with HCV genotype 1 in the study had baseline IP-10 levels between 150 and 600 pg/

Table 3. Multivariate logistic-regression to identify factors predictive of not achieving a sustained viral response ($n = 263$).

Parameter	Regression coefficient	SE	Odds ratio	*P*
Baseline $\log_{10}$ IP-10 level, pg/mL	1.2	0.43	3.318	.0049
Baseline $\log_{10}$ viral load, IU/mL	0.74	0.28	2.090	.0017
Age, years	0.03	0.01	1.031	.0475
HCV genotype				
1	0.76	0.27	0.356	.0059
2	−1.26	0.54	0.048	.0192
3	−1.29	0.42	0.046	.0020
Constant	−9.57	1.83		<.0001

NOTE. HCV, hepatitis C virus; IP-10, interferon-γ–inducible protein.

mL, which thus limits the predictive value of the baseline IP-10 level even in patients infected with HCV genotype 1. Similarly, almost all patients infected with HCV genotypes 2 and 3 in the study achieved an SVR, which limits the value of the baseline IP-10 level for predicting outcome in these patients. A recently completed treatment trial [32] demonstrated that patients infected with HCV genotypes 2 and 3 who received 16 weeks of treatment had slightly lower rates of SVR than those who received 24 weeks of treatment. In this setting, it will be of interest to examine whether IP-10 analysis may be useful in selecting patients who are suitable for shorter courses of treatment.

It is noteworthy that several of the factors previously known to be associated with an increased risk of failure of IFN treatment also were associated with baseline IP-10 levels—that is, HCV genotype 1 infection with high viral load, high BMI, and the presence of bridging fib osis or cirrhosis [5–8]. These find ings may be suggestive that IP-10 plays a role in the antiviral mechanism of action of IFN-α and ribavirin. However, considering the observation that IP-10 levels were lower in the majority of patients 6 weeks after the start of treatment and rebounded in patients who did not obtain SVR after the completion of treatment, it appears to be less probable that IP-10 is directly involved in the antiviral effect of combination treatment. More likely, the IP-10 level reflect the degree of local chemokine signaling in HCV-infected hepatocytes intended to recruit mononuclear cells to the liver to combat the ongoing viral infection, as indicated by the association between IP-10 levels and necroinflammato y activity in liver-biopsy samples (figu e 4). We hypothesize that as intrahepatic viral replication decreases during treatment, so does chemokine signaling from infected hepatocytes induced by viral replication. If the virus is not eradicated after the completion of treatment, intrahepatic viral replication—and, thus, chemokine signaling—resumes once antiviral treatment is discontinued. Obviously, we cannot exclude the possibility that the decrease in IP-10 levels noted 6 weeks after the start of treatment is a direct effect of IFN and ribavirin and is not only secondary to the decrease in viral load, especially given that the decrease in IP-10 levels was noted in all patients, even those without a virological response.

Harvey et al. [18] demonstrated an association between the expression of IP-10 mRNA in the liver and lobular necroinflammato y activity as scored by the Scheuer protocol [33] but not portal inflammatio or fib osis. By contrast, our results suggest a statistically significan association between IP-10 levels as measured in plasma and interface hepatitis, focal inflam mation, portal inflammation and fib osis stage as scored by the Ishak protocol [31]. This discrepancy may be due to differences in the methods for the quantificatio of IP-10 as well as in the protocols used for the scoring of liver-biopsy samples. The correlation between IP-10 levels at baseline and grade 1 steatosis in infection with HCV genotypes other than 3 may imply that this association might be secondary to an association between BMI and IP-10 levels. In line with this assumption, we have previously demonstrated an association between BMI and steatosis for HCV genotypes other than 3 [30]. The association between IP-10 levels and BMI is intriguing, but the underlying mechanisms remain unclear at present and warrant further investigation.

The correlation between IP-10 levels and necroinflammato y activity, fib osis stage, and viral load, as well as the significan decrease in IP-10 levels after the clearance of the HCV infection might imply that IP-10 plays a role in the natural pathogenesis of HCV-induced liver damage, as was suggested by Butera et al. [25]. Those researchers demonstrated that HCV-infected hepatocytes produce IP-10. Thus, it is feasible that, after IP-10 production by infected hepatocytes, larger numbers of inflam matory cells are recruited to the liver, as indicated by the association between IP-10 levels and necroinflammato y activity (figu e 4). If the infection is not overcome during the acute phase and becomes chronic, the inflammato y response will, over time, result in increasing fib otic changes. Indeed, it has previously been shown that long-standing necroinflammato y activity in the liver, especially in the form of interface hepatitis, is associated with an accelerated progression of fib osis [4].

In conclusion, we propose that pretreatment plasma IP-10 levels may play a role in predicting the outcome of combination antiviral treatment in patients infected with HCV genotypes 1 and 4. Thus, low circulating IP-10 levels before the onset of treatment were associated with a favorable initial reduction in serum viral loads, as well as with a sustained elimination of the virus after the completion of treatment. The prognostic value of IP-10, alone or in conjunction with other prognostic factors, should be further investigated, and studies should be performed aiming at definin the putative role of IP-10 in the resistance to antiviral treatment that is observed in many HCV-infected patients.

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References

1. Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science **1989**; 244:359–62.
2. Alter HJ, Purcell RH, Shih JW, et al. Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. N Engl J Med **1989**; 321:1494–500.
3. Saito I, Miyamura T, Ohbayashi A, et al. Hepatitis C virus infection is associated with the development of hepatocellular carcinoma. Proc Natl Acad Sci USA **1990**; 87:6547–9.

4. Lagging LM, Westin J, Svensson E, et al. Progression of fib osis in untreated patients with hepatitis C virus infection. Liver **2002**; 22:136–44.
5. Fried MW, Shiffman ML, Reddy KR, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med **2002**; 347:975–82.
6. Hadziyannis SJ, Sette H Jr, Morgan TR, et al. Peginterferon-α2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med **2004**; 140:346–55.
7. Manns MP, McHutchison JG, Gordon SC, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet **2001**; 358: 958–65.
8. Zeuzem S, Feinman SV, Rasenack J, et al. Peginterferon alfa-2a in patients with chronic hepatitis C. N Engl J Med **2000**; 343:1666–72.
9. Ferenci P. Predicting the therapeutic response in patients with chronic hepatitis C: the role of viral kinetic studies. J Antimicrob Chemother **2004**; 53:15–8.
10. Tsubota A, Arase Y, Someya T, et al. Early viral kinetics and treatment outcome in combination of high-dose interferon induction vs. pegylated interferon plus ribavirin for naive patients infected with hepatitis C virus of genotype 1b and high viral load. J Med Virol **2005**; 75:27–34.
11. Luster AD, Unkeless JC, Ravetch JV. γ-Interferon transcriptionally regulates an early-response gene containing homology to platelet proteins. Nature **1985**; 315:672–6.
12. Neville LF, Mathiak G, Bagasra O. The immunobiology of interferon-gamma inducible protein 10 kD (IP-10): a novel, pleiotropic member of the C-X-C chemokine superfamily. Cytokine Growth Factor Rev **1997**; 8:207–19.
13. Taub DD, Sayers TJ, Carter CR, Ortaldo JR. Alpha and beta chemokines induce NK cell migration and enhance NK-mediated cytolysis. J Immunol **1995**; 155:3877–88.
14. Taub DD, Lloyd AR, Conlon K, et al. Recombinant human interferon-inducible protein 10 is a chemoattractant for human monocytes and T lymphocytes and promotes T cell adhesion to endothelial cells. J Exp Med **1993**; 177:1809–14.
15. Loetscher M, Gerber B, Loetscher P, et al. Chemokine receptor specifi for IP10 and Mig: structure, function, and expression in activated T-lymphocytes. J Exp Med **1996**; 184:963–9.
16. Weng Y, Siciliano SJ, Waldburger KE, et al. Binding and functional properties of recombinant and endogenous CXCR3 chemokine receptors. J Biol Chem **1998**; 273:18288–91.
17. Hua LL, Lee SC. Distinct patterns of stimulus-inducible chemokine mRNA accumulation in human fetal astrocytes and microglia. Glia **2000**; 30:74–81.
18. Harvey CE, Post JJ, Palladinetti P, et al. Expression of the chemokine IP-10 (CXCL10) by hepatocytes in chronic hepatitis C virus infection correlates with histological severity and lobular inflammation J Leukoc Biol **2003**; 74:360–9.
19. Tsunoda I, Lane TE, Blackett J, Fujinami RS. Distinct roles for IP-10/CXCL10 in three animal models, Theiler's virus infection, EAE, and MHV infection, for multiple sclerosis: implication of differing roles for IP-10. Mult Scler **2004**; 10:26–34.
20. Liu MT, Keirstead HS, Lane TE. Neutralization of the chemokine CXCL10 reduces inflammato y cell invasion and demyelination and improves neurological function in a viral model of multiple sclerosis. J Immunol **2001**; 167:4091–7.
21. Christen U, Von Herrath MG. IP-10 and type 1 diabetes: a question of time and location. Autoimmunity **2004**; 37:273–82.
22. Nicoletti F, Conget I, Di Mauro M, et al. Serum concentrations of the interferon-γ-inducible chemokine IP-10/CXCL10 are augmented in both newly diagnosed type I diabetes mellitus patients and subjects at risk of developing the disease. Diabetologia **2002**; 45:1107–10.
23. Kolb SA, Sporer B, Lahrtz F, Koedel U, Pfiste HW, Fontana A. Identificatio of a T cell chemotactic factor in the cerebrospinal flui of HIV-1-infected individuals as interferon-γ inducible protein 10. J Neuroimmunol **1999**; 93:172–81.
24. Kutsch O, Oh J, Nath A, Benveniste EN. Induction of the chemokines interleukin-8 and IP-10 by human immunodeficienc virus type 1 tat in astrocytes. J Virol **2000**; 74:9214–21.
25. Butera D, Marukian S, Iwamaye AE, et al. Plasma chemokine levels correlate with the outcome of antiviral therapy in patients with hepatitis C. Blood **2005**; 106:1175–82.
26. Diago M, Castellano G, Garcia-Samaniego J, et al. Association of pretreatment serum interferon γ inducible protein 10 levels with sustained virological response to peginterferon plus ribavirin therapy in genotype 1 infected patients with chronic hepatitis C. Gut **2006**; 55:374–9.
27. Zeuzem S, Pawlotsky JM, Lukasiewicz E, et al. International, multicenter, randomized, controlled study comparing dynamically individualized versus standard treatment in patients with chronic hepatitis C. J Hepatol **2005**; 43:250–7.
28. Westin J, Lagging LM, Spak F, et al. Moderate alcohol intake increases fib osis progression in untreated patients with hepatitis C virus infection. J Viral Hepat **2002**; 9:235–41.
29. Westin J, Lagging LM, Wejstal R, Norkrans G, Dhillon AP. Interobserver study of liver histopathology using the Ishak score in patients with chronic hepatitis C virus infection. Liver **1999**; 19:183–7.
30. Westin J, Nordlinder H, Lagging M, Norkrans G, Wejstal R. Steatosis accelerates fib osis development over time in hepatitis C virus genotype 3 infected patients. J Hepatol **2002**; 37:837–42.
31. Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. J Hepatol **1995**; 22:696–9.
32. Shiffman ML, Pappas S, Nyberg L, et al. Peginterferon alpha-2a (Pegasys) plus ribavirin (Copegus) for 16 or 24 weeks in patients with HCV genotype 2 or 3: fina results of the ACCELERATE trial. J Hepatol **2006**; 44(Suppl 1):S271.
33. Scheuer PJ. Classificatio of chronic viral hepatitis: a need for reassessment. J Hepatol **1991**; 13:372–4.